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(+)- and (−)-Petromyroxols: Antipodal Tetrahydrofurandiols from Larval Sea Lamprey (*Petromyzon marinus* L.) That Elicit Enantioselective Olfactory Responses

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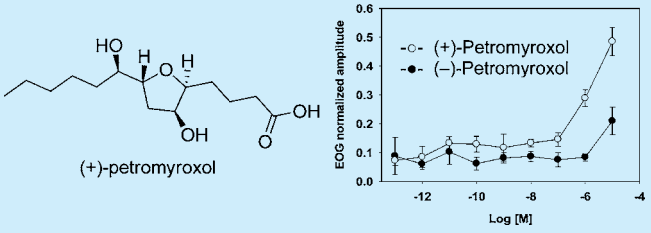
Supporting Information

ABSTRACT: (+)- and (−)-Petromyroxol [(+)-**1** and (−)-**1**, respectively], two novel tetrahydrofuran (THF)-diol fatty acid enantiomers, were isolated from water conditioned with larval sea lamprey. We herein describe their isolation and subsequent resolution using chiral chromatography. The absolute configuration of each enantiomer was determined by a combination of Mosher ester analysis and comparison with related natural and synthetic products. Electro-olfactogram (EOG) assays indicated that (+)-petromyroxol (**1**) possesses potent olfactory activity for sea lamprey.



A functionalized THF ring characterizes a plethora of natural products with a wide variety of biological properties, such as antitumor, antimalarial, antimicrobial, immunosuppressant, antifeedant, pesticidal, and pheromone activities.[1] Many of these compounds belong to the large family known as acetogenins.[2] Moreover, compounds with a THF−diol moiety have been shown to inhibit development and reproduction in nematodes and rats.[1,3] Compounds of this class also stimulate proliferation of human breast and prostate cancer cells.[3c,d,4] Finally, the four stereoisomers of 2-hexyl-4-acetoxytetrahydrofuran have recently been reported to have different odor properties and intensity.[5]

Our laboratories are involved in the identification of compounds with pheromone activity in the sea lamprey (*Petromyzon marinus*), an invasive vertebrate of the Laurentian Great Lakes.[6] In our investigation into the bioactive secondary metabolites of the lamprey, we have isolated and characterized a pair of novel fatty acid enantiomers, herein named (+)- and (−)-petromyroxol [(+)-**1** and (−)-**1**], having the constitution shown as structure **X** in Figure 1. Compounds (monoacetogenins) with a THF−diol subunit are known to occur in terrestrial plants,[7] marine algae,[8] and marine mollusks.[9] The petromyroxol enantiomers represent the first examples of THF−diols isolated from a vertebrate animal.

The THF−diol **X** (Figure 1) can exist as 16 possible stereoisomers. The relative configuration of petromyroxol (**1**) was elucidated using 2D NMR spectroscopy and confirmed by comparison with natural and synthetic analogs (see Supporting Information). To evaluate the biological activity of each enantiomer of the petromyroxols, we separated them by chiral chromatography. The absolute configuration of each enantiomer was determined by Mosher ester analysis. The enantiomeric ratio (*er*) of the natural sample was measured. Here we describe the isolation, structure elucidation, determination of the *er*, and olfactory potency of (+)- and of (−)-petromyroxol [(+)-**1** and (−)-**1**].

Initial screening of the extract of >100 000 L of sea lamprey larvae-conditioned water indicated that fractions eluting from silica gel in ca. 6−8:1 ($CHCl_3$/MeOH) induced the highest electro-olfactogram (EOG) responses in adult sea lamprey. Further EOG-guided fractionation and purification yielded petromyroxol (**1**) as a yellowish oil with a specific rotation of $[\alpha]^{25}_{D}$ −4.0 (*c* 0.10, MeOH) (2.90 mg). One molecular formula suggested by high resolution mass spectrometry (*m*/*z* 273.1709 $[M - H]^-$, ΔmDa 0.7, and 297.1684 $[M + Na]^+$, ΔmDa 0.6) was $C_{14}H_{26}O_5$, which implied two degrees of unsaturation.

Through iterative analyses of the battery of 1D and 2D NMR spectral data (below) we deduced the constitution of **X** (Figure 1). The ^{1}H NMR (500 MHz) spectrum (Supporting Information) showed signals for one methyl (H-14), four methines (H-5, H-6, H-8, and H-9), and eight methylenes (H-2 ∼ H-4, H-7, and H-10−H-13). The proton chemical shifts (Table 1) and formula suggested by the mass spectra indicated that the four methine carbons were oxygenated. The methylene resonances overlapped from δ_H 1.2 to 1.7 ppm, implicating the presence of an aliphatic chain. The ^{13}C NMR (Table 1, 125 MHz) and HSQC (Supporting Information) spectra revealed

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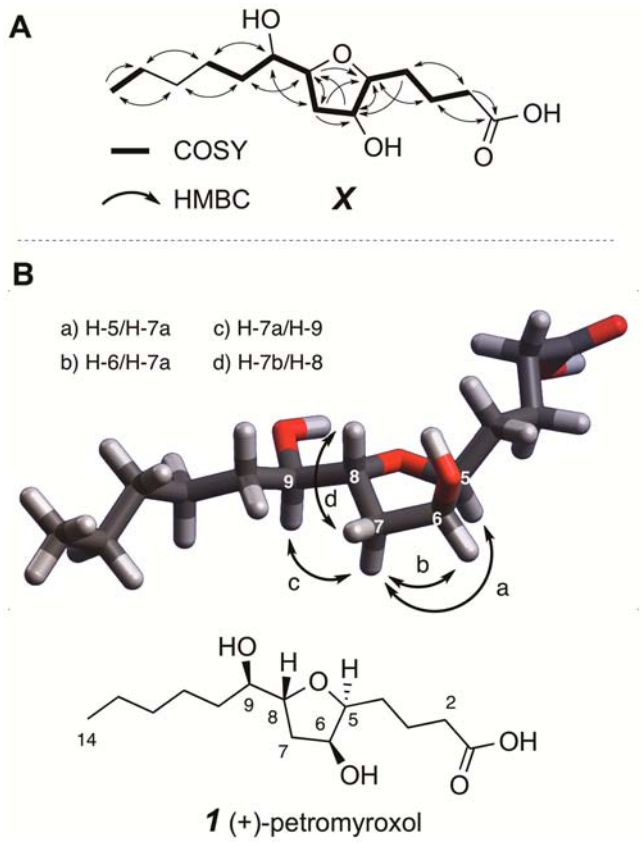

Figure 1. (A) Key COSY and HMBC (proton to carbon) correlations. (B) NOESY correlations from which the constitution and relative configuration of (+)- and (−)-petromyroxol were deduced.

Table 1. NMR Spectroscopic Data (500 and 125 MHz, $CDCl_3$) for Petromyroxol (1)

C no.	δ_C, type	δ_H, mult (J in Hz)
1	177.6, C	–
2	33.7, CH_2	2.43 m (ΣJs = 18)
3	21.4, CH_2	1.77 m, 1.70 m
4	28.4, CH_2	1.72 m, 1.67 m
5	82.5, CH	3.79 ddd (ca. 2.5, 6.5, 6.5)
6	73.5, CH	4.30 dd (ca. 3.5, 3.5)
7a	37.8, CH_2	1.89 ddd (4.6, 9.2, 13.7)
7b		2.02 dd (6.6, 13.4)
8	80.7, CH	4.06 ddd (6.5, 6.5, 8.9)
9	74.3, CH	3.39 m (ΣJs = 18)
10	33.3, CH_2	1.40 m
11	25.4, CH_2	1.51 m, 1.38 m
12	32.0, CH_2	1.29 m
13	22.8, CH_2	1.31 m
14	14.2, CH_3	0.89 t (6.9)

the presence of 14 carbon signals corresponding to one carbonyl, four methine, one methyl, and eight methylene carbons. The four deshielded resonances (δ_C 73−83 ppm) further supported the presence of four oxygenated methine carbons. The $^1H-^1H$ COSY correlations suggested the relative spacing (i.e., two vicinal pairs separated by a methylene group) among the four oxygenated aliphatic carbons.

To account for the second degree of unsaturation (in addition to the carbonyl), the presence of a cyclic ether was deduced. HMBC correlations from H-6 to C-8 and C-5; from H-7a and H-7b to C-5, C-8, and C-6; and from H-8 to C-5, C-6, and C-7 established the presence of a tetrahydrofuranyl ring between C-5 and C-8. The chemical shifts of H-6 and C-6 (δ_H 4.28; δ_C 73.3) and $^1H-^1H$ COSY correlations confirmed the presence of a hydroxy group on C-6.

The presence of a 4-substituted butyric acid moiety was indicated by $^1H-^1H$ COSY correlations between H_2-2/H_2-3 and H_2-3/H_2-4 and by HMBC correlations from H_2-2 to C-1, C-3, and C-4 and from H_2-3 to C-1, C-2, and C-4 (Figure 1). The connection of C-4 to C-5 was indicated by the associated COSY correlations of H_2-4/H-5 and HMBC correlations from H_2-4 to C-6; from H_2-3 to C-5; and from H-5 to C-3. The remaining proton and carbon resonances were attributable to a tetrahydrofuranyl ring. The key $^1H-^1H$ COSY correlations of H-8/H-9 and HMBC correlations from H_2-7 to C-9; from H-8 to C-10; from H-9 to C-7; and from H_2-10 to C-8, indicated the hydroxy group substitution at C-9 and supported the linkage between C-8 and C-9. Combining the three substructures, a 4-substituted butyric acid, a 3-hydroxy-2,5-disubstituted tetrahydrofuran, and an α-hydroxyhexyl, gives rise to the constitution of **X**.

The relative configuration of petromyroxol (**1**) was initially established by analyses of the NOESY correlations as summarized in Figure 1B. NOESY correlations between H-5/H-6, H-6/H-7a, and H-5/H-7a placed these protons on the same face of the THF ring. We then located the report of a natural product with a substructure sharing this relative configuration.[3b,10] The reported proton and carbon NMR chemical shifts showed a strong similarity with those of **1**.[3a,b,10]

The isolated sample of petromyroxol (**1**) showed a specific optical rotation of $[\alpha]^{25}_D$ −4.0 (*c* 0.10, MeOH). To our surprise, analysis by chiral HPLC (Diacel Chiralpak AD-H, APCI MS/MS detection) showed that the sample comprised a substantial amount of both enantiomers of **1** (Figure 2). The *er* [(+)/(−)] of

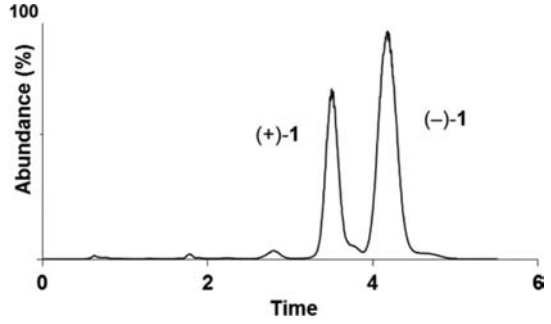

Figure 2. Analyses of (+)- and (−)-petromyroxol by chiral HPLC-MS/MS in APCI positive mode.

the natural petromyroxol was estimated to be ca. 36:64, as deduced from the peak integration of the chromatogram (Figure 2). This material was separated (3 injections) to provide the faster and slower eluting antipodes (0.912 and 1.50 mg, respectively). Coincidentally, these were measured to have the same absolute value of specific rotation, namely, $[\alpha]^{25}_D$ = +17.0 (*c* 0.36, $CHCl_3$) and −17.0 (*c* 0.36, $CHCl_3$), respectively. These values are in close accord with the observed rotation of the initial isolated natural sample (i.e., $[\alpha]^{25}_D$ −4.0).

The absolute configuration of (−)-petromyroxol was then determined by a Mosher ester analysis.[11] Treatment of (−)-**1** with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA-Cl] in pyridine yielded a mixture of the 6,9-

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bis(*S*)-Mosher ester and 6-mono-(*S*)-Mosher ester of (−)-**1**. Similar treatment of (−)-**1** with (*S*)-(+)-MTPA-Cl afforded an analogous mixture of the bis- and mono-(*R*)-Mosher esters. Each of these mixtures was separated by semipreparative HPLC to obtain the mono-(*S*)- and mono-(*R*)-Mosher ester, respectively. The ($\Delta\delta_{S\text{-}R}$) chemical shift values are summarized in Figure 3, from which the configuration at C-6 was deduced to be *R*. Therefore, (−)-**1** exhibits the 5*R*,6*R*,8*S*,9*S*-configuration and, by inference, (+)-**1** is the 5*S*,6*S*,8*R*,9*R*-antipode.

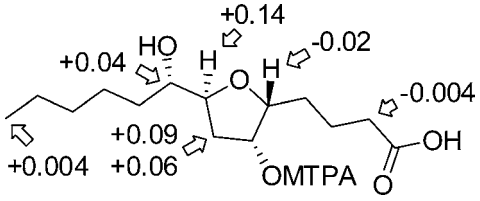

Figure 3. $\Delta\delta_{S\text{-}R}$ values for the mono-Mosher esters of (−)-**1**.

We then used an EOG assay to evaluate the olfactory response of adult sea lamprey to each enantiomer of petromyroxol (**1**). In the context of lamprey olfaction and pheromone communication, odorants that trigger conspecific physiological functions can have an EOG detection threshold concentration as low as 10^{-12} M.[12] Active agents that induce behavioral responses within the range of 10^{-11}−10^{-14} M are also known.[13] The petromyroxol enantiomer with a positive specific rotation value [i.e., (+)-**1**] elicited a stronger olfactory response than the (−)-enantiomer [(−)-**1**] in the concentration range of 10^{-8} to 10^{-6} M (Figure 4).

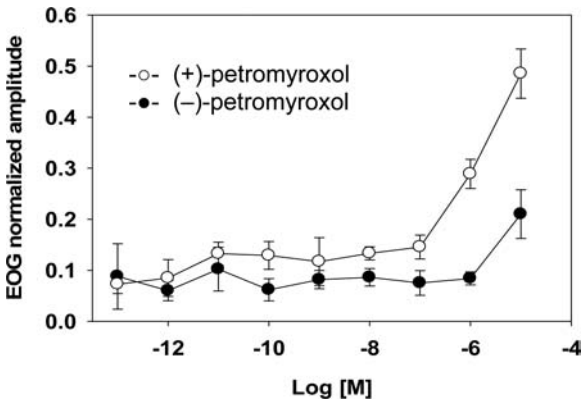

Figure 4. Semilogarithmic plot of mean (±S.E.) pooled normalized electro-olfactogram (EOG) amplitudes recorded in adult lamprey in response to (+)-petromyroxol [(+)-**1**] and (−)-petromyroxol [(−)-**1**]. $n = 7$.

The detection thresholds were estimated by fitting the concentration/response data to a logistic regression model[14] and determining the lowest concentration at which a response different from the baseline was elicited. Only the data for (+)-**1** fit the model [with a threshold of detection at 10^{-11} M ($P < 0.01$)], which is consistent with interactions between an olfactory odorant and its cognate receptors.[14−16] Response data for (−)-**1** did not fit the model. Whether (+)-petromyroxol [(+)-**1**] is a component of a pheromone mixture in the sea lamprey needs to be determined through additional biological experiments.

In summary, (+)- and (−)-petromyroxol, enantiomeric fatty acids with an embedded THF−diol moiety, were isolated and characterized from water conditioned with larval sea lamprey. These enantiomers represent the first examples of such fatty acid derivatives. Moreover, (+)-petromyroxol showed a highly potent olfactory activity, which could be developed as an effective odorant for further research to understand chemical communication in sea lamprey.

■ ASSOCIATED CONTENT

Supporting Information

Procedures for isolation of the natural sample. Structures of reference compounds used to confirm structural assignments. NMR (^{1}H, ^{13}C, gCOSY, HSQC, and HMBC) and HR-MS data for the natural sample of petromyroxol (**1**). ^{1}H NMR and gCOSY data of the mono-Mosher esters of (−)-**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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